Use of decapsulated *Artemia* cysts in ornamental fish culture

L C Lim[1], Y L Cho[1], P Dhert[2], C C Wong[1], H Nelis[3] & P Sorgeloos[2]

[1]Freshwater Fisheries Centre, Agri-food and Veterinary Authority of Singapore, 17 km Sembawang Research Station, Lorong Chencharu, Singapore 769194, Republic of Singapore

[2]Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Gent, Belgium

[3]Laboratory of Pharmaceutical Microbiology, Harelbekestraat 72, Gent, Belgium

Correspondence: Lian Chuan Lim, Freshwater Fisheries Centre, Agri-food and Veterinary Authority of Singapore, Sembawang Research Station, Lorong Chencharu, Singapore 769194, Republic of Singapore. E-mail: 333@post1.com

Abstract

Two series of feeding experiments were conducted to study the feasibility of using decapsulated *Artemia* cysts for direct feeding to ornamental fish. The first series evaluated the dietary values of the dried and the brine decapsulated *Artemia* cysts against two conventional live feeds, *Artemia* nauplii and *Moina* for adults and fry of the guppy, *Poecilia reticulata* Peters. In the second series, brine cysts were used for feeding to fry of four other important ornamental fish species, viz. platy, *Xiphophorus maculatus* (Günther), swordtail, *X. helleri* (Heckel), molly, *P. sphenops* Cuvier & Valenciennes and black neon tetra, *Hyphessobrycon herbertaxelrodi* Géry, and the results were compared with those fed *Moina*. Evaluation of the performance of the fish fed the various diets was based on stress resistance, growth and survival of the fish. Our findings indicated that decapsulated cysts could be used as a substitute for *Artemia* nauplii or *Moina* in freshwater ornamental fish culture. Apart from being a hygienic off-the-shelf feed, the direct use of the cysts also signifies a new area of application for low-hatch cysts in the ornamental fish industry, with concomitant saving in feed costs.

Keywords: decapsulated *Artemia* cysts, guppy, swordtail, molly, tetra, platy

Introduction

In freshwater ornamental fish culture, *Moina* used to be the most common live food organism for feeding young fish in the industry. As *Moina* is cultured in water enriched with organic manure, an increasing number of ornamental fish farmers have shifted from the use of potentially contaminated *Moina* to *Artemia* nauplii for feeding their fish (Lim, Soh, Dhert & Sorgeloos 2001). However, the high cost of *Artemia* cysts has increased fish production costs and cheaper alternative diets with similar nutritional quality are needed to maintain the cost competitiveness of the fish in the global market. Because a considerable amount of *Artemia* cysts in the world market have a low commercial value as a result of their low hatching rate (Ribeiro & Jones 1998), they could easily be transformed to useful feed for freshwater ornamental fish and result in savings to the industry.

Artemia cysts consist of dormant *Artemia* embryos covered with a three-layered cyst shell. Under optimal hatching conditions, the embryos break out of the shell and hatch into nauplii, which are used for larval or fry feeding of species with a high commercial value. The hard outer shell of the *Artemia* cyst, the alveolar layer, can be completely removed through a chemical process known as decapsulation (Sorgeloos, Bossuyt, Lavens, Leger, Vanhaecke & Versichele 1983). The decapsulated cysts can then be used immediately, or dehydrated in brine solution for storage (brine cysts), or further subjected to a drying process for longer term storage (dried cysts). The advantages of decapsulation include disinfection of the cysts, improved hatching of the cysts into nauplii, higher energy content of the nauplii, and no risk of fish larvae suffering from gut obstruction due to the

ingestion of the cyst shell (Bengtson, Leger & Sorgeloos 1991).

An important application of the decapsulated cysts is direct feeding to the predator, in which case even low-hatch or no-hatch cysts can be used for feeding. Sorgeloos, Bossuyt, Lavina, Baeza-Mesa & Persoone (1977) first suggested the use of decapsulated cysts as a direct source for fish and crustacean larvae. Subsequent studies demonstrated that decapsulated cysts are a good feed similar to freshly hatched *Artemia* nauplii for the larvae of marine shrimps and freshwater prawn, such as *Metapenaeus monoceros* (Fabricius) (Royan 1980), *Penaeus monodon* Fabricius (Mock, Fontaine & Revera 1980), *P. indicus* H. Milne Edwards, *M. ensis* de Haan, *M. endeavouri* (Schmitt) and *Macrobrachium rosenbergii* (De Man) (Bruggeman, Sorgeloos & Vanhaecke 1980). For fish species, De Los Santos, Sorgeloos, Lavina & Bernardino (1980) reported that the larvae of milkfish, *Chanos chanos* (Forsskal) grew from 0.024 to 5 g with 80–95% survival when fed decapsulated cysts, but no further data were available. Verreth, Storch & Segner (1987) studied the nutritional quality of several dry diets for larvae of African catfish, *Clarias gariepinus* (Burchell), and found that dried decapsulated *Artemia* cysts gave the best growth and survival rate consistently, but there was no comparison with those of fish fed live feed. Similarly, larvae of the catfish fed dried cysts, including cysts enriched with vitamin C, also recorded significantly faster growth than those fed brine cysts, freshly decapsulated cysts or microbound diet (Pector, Tackaert, Abelin, Ollevier & Sorgeloos 1994; Bardocz, Kovacs, Radics & Sandor 1999). Despite these positive results, commercial application of direct feeding of decapsulated cysts has so far been restricted to only some shrimp hatcheries. In this paper, we evaluated the dietary values of two forms of decapsulated cysts, viz. brine cysts and dried cysts, for ornamental fish culture. Five groups of freshwater ornamental fish, viz. guppy, *Poecilia reticulata* Peters, platy, *Xiphophorus maculatus* (Günther), swordtail, *X. helleri* (Heckel), molly, *P. sphenops* Cuvier & Valenciennes, and tetra (represented by black neon tetra, *Hyphessobrycon herbertaxelrodi* Géry) were tested. These five groups of fish made up to 35% of the fish exports from Singapore in 2000 (Agri-food & Veterinary Authority of Singapore, unpublished data).

Materials and methods

Experimental design

Two series of feeding experiments were designed in this study. In the first series, two experiments using fry and adults of the guppy, *P. reticulata*, respectively, were conducted to evaluate the dietary values of brine cysts and dried cysts. The results were compared with those of fish fed the two conventional live feeds, *Artemia* nauplii and *Moina* in 4-week feeding experiments. The second series of experiments was designed to evaluate the dietary value of brine cysts against that of *Moina* for fry of other common ornamental fish species. The series consisted of four feeding experiments of 3 weeks using the fry of platy, *X. maculatus*, swordtail, *X. helleri*, molly, *P. sphenops* and tetra (represented by black neon tetra, *H. herbertaxelrodi*). All the experiments were run using four replicates for each treatment group.

Feed sources

All Artemia used in this experiment originated from Great Salt Lake (UT, USA). The cysts used for preparation of decapsulated cysts were of low hatchability. They were decapsulated according to the technique described in Bengtson *et al.* (1991). This process involved hydration of the cysts, removal of the shell with an alkaline hypochlorite solution, and washing and deactivation of the hypochlorite. After decapsulation, the cysts were dehydrated in a saturated brine solution for storage (= brine cysts). To prepare dried cysts, a portion of the brine cysts was rinsed and dried at 40 °C in a fluidized bed dryer until individual cysts were obtained. These dried cysts have a longer shelf life and better buoyancy in water than the brine cysts. Moina was acquired from a commercial Moina farm in Malaysia. *Artemia* nauplii were hatched using the standard procedures described in Sorgeloos, Lavens, Leger, Tackert & Versichele (1986). They were harvested 21–22 h after incubation as Instar I nauplii and either fed to the fish immediately or stored at 4 °C until the time for feeding.

Rearing system

All six experiments were conducted in 50-L glass aquaria (0.60 m × 0.30 m × 0.36 m), each

containing 40 L of aged tap water. Each aquarium was equipped with a submerged box bio-filter and provided with aeration. All the fish used in this study were collected from the hatchery or production facilities of commercial farms in Singapore. Except for guppy fry that were stocked at 200 fry per aquarium, the guppy adults and the fry of the four other species were stocked at 100 fish per aquarium. The fry of all the species used in this study were 1-day old (mean total length, TL = 8.5–9.6 mm), with the exception of tetra. For tetra, 11-day-old fry (12.8 mm TL) were used instead, as this was the age of fry that were ready to feed on *Artemia* cysts. The guppy adults used in this study were 2-month old (22.7 mm TL). All the fish were fed *ad libitum* three times a day at 09:00, 12:00 and 15:30 hours respectively. Before the first feeding, the aquaria were cleaned and one-third of the water was changed daily. The water quality was also monitored weekly to ensure optimal experimental conditions so as to attribute any parameter differences to the diets solely. The water quality parameters that were monitored were temperature (range 26.0–27.5 °C), pH (6.4–6.6), dissolved oxygen (7.5–7.9 mg L^{-1}), ammonia (< 0.02 mg L^{-1}) and nitrite (< 0.1 mg L^{-1}), and all were found to be within optimal ranges for the fish tested, with no significant differences in these water quality parameters among the aquaria.

Monitoring of fish performance

To monitor the effects of the various diets on fish performance, stress resistance, survival and growth (dry weight, wet weight and total length) were determined weekly for all the aquaria, starting from the first week after feeding in guppy adult and 2 weeks after feeding in the fry of all the five fish species. At the end of each week, a sample of 10 fish was taken from each aquarium for stress test, which was followed by measurements of wet weight and dry weight. For wet weight measurements, 10 fish were dried on a towel paper and weighed to the nearest 0.1 mg. Total length was measured to the nearest 0.1 mm under a binocular microscope. The fish were then dried in an oven at 60 °C for 24 h and weighed again for dry weight. Dead fish were counted and removed from individual aquariums daily before the first feeding to determine survival rates. In order to obtain the exact survival rate, the final number of fish at the end of each week was corrected for the number of sampled fish to which a mortality rate was attributed according to the measured mortality distribution.

Stress test

A stress test was used to evaluate the stress resistance of the freshwater ornamental fish, according to the procedure described by Lim, Wong, Koh, Dhert & Sorgeloos (2000). The test entailed exposure of the fish to osmotic shock in a saline solution, and the mortality of the fish was monitored at 3-min intervals over a 2-h period. The stress resistance of the fish is expressed as stress index, which is obtained as the sum of 40 cumulative mortality readings recorded during the observation period. Higher index, a result of either earlier or higher mortality or both, indicates a lower stress resistance of the fish and *vice versa*. As the resistance to osmotic shock varied with species and the stage of development, preliminary tests involving a wide range of salinities were conducted to determine the optimal salinity for the stress test for the respective species under study, with the exception of guppy adults and fry. The optimal salinities for stress tests using guppy and guppy fry were reported to be 35‰ and 30‰ respectively (Lim *et al.* 2000). Determination of optimal salinity was based on the mortality pattern of the fish during a 2-h period, and the salinity that resulted in the longest period between the first and last fish mortality was selected. These periods ranged from 48 min for platy fry to 75 min for molly fry. The optimal salinities were found to be 35‰ for molly fry, 25‰ for platy fry, 20‰ for swordtail fry and 15‰ for tetra fry (Fig. 1). These salinities were used for all the stress tests for the respective fish in the subsequent experiments. For swordtail fry at 3 weeks after feeding, 25‰ was used, as 20‰ was too low to cause significant mortality of the swordtail fry, due to an increased tolerance to the salinity stress in the older fry.

Biochemical analyses of diets and fish tissue

For all the fry experiments, fish specimens collected from the four aquaria of each treatment group were pooled together. Two samples for biochemical analysis were collected from each treatment group. All the 24 fish samples, together with eight feed samples (two for each feed), were preserved in an ultra-low freezer at −80 °C until biochemical analyses. Fatty

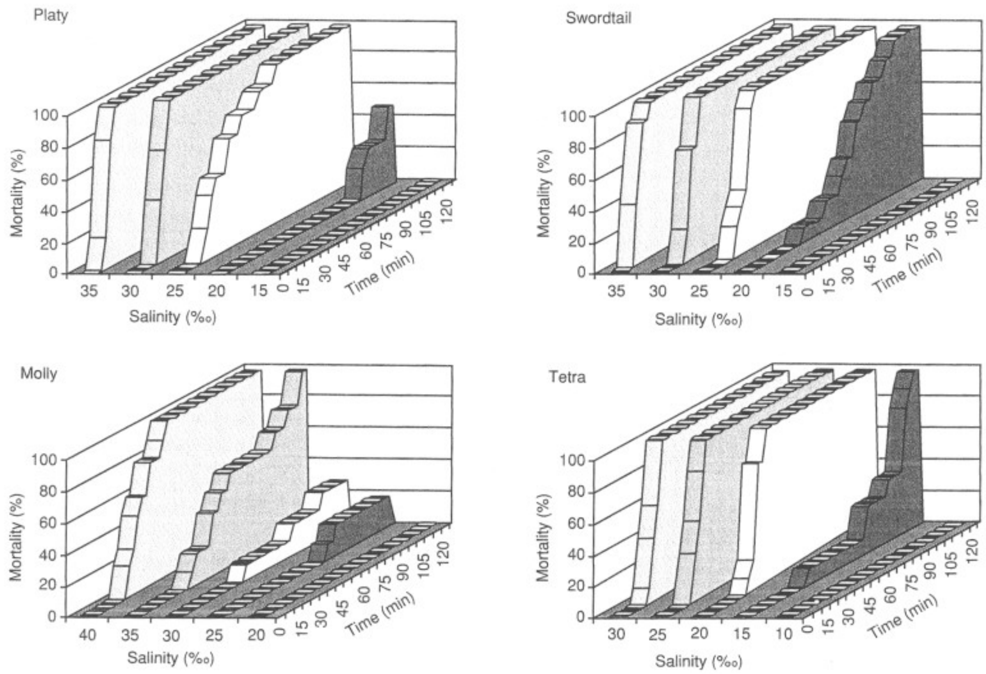

Figure 1 Determination of the optimal salinities for stress test on fry of platy, *Xiphophorus maculatus* (Günther), swordtail, *X. helleri* (Heckel), molly, *P. sphenops* Cuvier and tetra, *Hyphessobrycon herbertaxelrodi* Géry.

Acid Methyl Ester (FAME) analyses were performed according to the method modified from Lepage & Roy (1984) (Coutteau & Sorgeloos 1995). Ascorbic acid (AA) contents were determined by high-performance liquid chromatography and electrochemical detection according to the procedure described by Nelis, Merchie, Lavens, Sorgeloos & Leenheer (1997).

Statistical analysis

The mean and standard deviation were calculated for all five parameters in each treatment group. Results were subjected to one-way analysis of variance followed by Tukey's all comparison test ($P < 0.05$) or the Student's *t*-test ($P < 0.01$ or 0.05), using a software package Statistica version 5.0 (StatSoft, Inc., Tulsa, OK, USA) to determine significant differences among treatment means.

Results

Performance of guppy adults

Although the decapsulated *Artemia* cysts (240 μm in diameter) were quite small for the size of the guppy adults (mean = 22.7 mm TL), they were eagerly consumed by the fish. The performance of guppy adults fed decapsulated *Artemia* cysts and live feeds is presented in Table 1. During the early period, the two groups of cyst-fed guppy, especially that fed brine cysts, showed a higher stress resistance than fish fed *Moina*. This effect became less pronounced towards the end of the experimental period. After 4 weeks of feeding, there was no significant difference in stress resistance among the four treatments. For survival rate and growth performance in terms of total length, wet weight and dry weight, no significant differences could be detected between the two groups of cyst-fed fish throughout the experimental period. Their performance was similar to the group fed *Artemia* nauplii and significantly better than that fed *Moina*.

Performance of guppy fry

Guppy fry showed good feeding response to both brine cysts and dried cysts. They eagerly consumed the cysts, even after the cysts had sunken to the aquarium bottom. The performance of guppy fry fed four different diets is shown in Table 2. The effects of diet on stress resistance of the guppy fry were more pronounced than for guppy adults. After 4 weeks of feeding, guppy fry fed either brine cysts or

Table 1 Performance of guppy, *Poecilia reticulata* Peters, adults fed different diets

Parameters and duration of feeding	Brine cysts	Dried cysts	*Artemia* nauplii	*Moina*
Stress index (at 35‰)				
1 week	227.0 ± 12.03[a]	244.5 ± 2.38[a]	241.5 ± 8.66[a]	264.8 ± 9.25[b]
2 weeks	82.8 ± 18.36[a]	142.3 ± 13.57[b]	136.8 ± 11.62[b]	162.0 ± 14.45[b]
3 weeks	232.5 ± 11.70[a]	251.5 ± 10.12[a,b]	244.5 ± 6.25[a,b]	259.8 ± 8.66[b]
4 weeks	225.5 ± 4.80[a]	242.8 ± 9.91[a]	233.5 ± 17.75[a]	246.5 ± 7.33[a]
Wet weight (mg)[2]				
1 week	184.7 ± 16.45[a,b]	198.0 ± 6.95[a]	172.4 ± 19.26[a,b]	156.7 ± 9.50[b]
2 weeks	237.3 ± 15.96[a,b]	245.2 ± 16.30[a]	236.2 ± 11.36[a,b]	212.9 ± 10.11[b]
3 weeks	278.1 ± 10.49[a]	290.7 ± 16.76[a]	279.6 ± 11.18[a]	221.0 ± 10.66[b]
4 weeks	323.3 ± 19.06[a]	343.9 ± 15.48[a]	323.1 ± 14.43[a]	260.3 ± 15.76[b]
Dry weight (mg)				
1 week	60.6 ± 3.63[a]	68.3 ± 4.06[a]	60.1 ± 5.94[a,b]	51.4 ± 3.34[b]
2 weeks	95.9 ± 6.82[a]	101.0 ± 5.44[a]	92.7 ± 5.11[a]	70.1 ± 4.04[b]
3 weeks	108.4 ± 5.09[a]	115.2 ± 7.96[a]	109.6 ± 5.04[a]	77.0 ± 5.50[b]
4 weeks	112.6 ± 10.97[a]	119.5 ± 6.12[a]	110.1 ± 2.62[a]	78.0 ± 2.44[b]
Total length (mm)[2]				
1 week	24.2 ± 0.46[a,b]	24.7 ± 0.13[a]	24.2 ± 0.61[a,b]	23.7 ± 0.36[b]
2 weeks	26.3 ± 0.68[a]	26.5 ± 0.53[a]	26.2 ± 0.21[a]	25.2 ± 0.29[b]
3 weeks	28.8 ± 0.39[a]	29.1 ± 0.54[a]	28.5 ± 0.35[a]	27.2 ± 0.55[b]
4 weeks	30.2 ± 0.25[a]	31.1 ± 0.53[a]	30.2 ± 0.52[a]	29.0 ± 0.62[b]
Survival rate (%)				
1 week	100.0 ± 0[a]	99.8 ± 0.50[a]	100.0 ± 0[a]	99.0 ± 1.15[a]
2 weeks	100.0 ± 0[a]	99.8 ± 0.50[a]	100.0 ± 0[a]	98.3 ± 1.26[b]
3 weeks	100.0 ± 0[a]	99.8 ± 0.50[a]	100.0 ± 0[a]	98.3 ± 1.26[b]
4 weeks	100.0 ± 0[a]	99.8 ± 0.50[a]	99.5 ± 1.00[a]	98.0 ± 0.82[b]

Values are means ± standard deviations from four replicates. Values within each row that do not share the same superscript are significantly different ($P < 0.05$). Initial fish weight and length were 148.0 mg and 22.7 mm respectively.

dried cysts displayed significantly higher stress resistance than those fed either *Artemia* nauplii or *Moina*. For growth performance, guppy fry fed dried cysts grew significantly faster than those fed *Artemia* nauplii. The growth of fish fed brine cysts was similar to those fed *Artemia* nauplii, with no significant difference between the two groups. When compared with *Moina*, both dried cysts and brine cysts also fared significantly better in terms of dry weight and wet weight. Of the two cyst-fed groups, only guppy fry fed brine cysts had a significantly higher survival than those fed either *Artemia* nauplii or *Moina*. The survival rate of fry fed dried cysts did not differ significantly from that fed either *Artemia* nauplii or *Moina*.

Between the two groups of cyst-fed guppy fry, the fish fed brine cysts displayed consistently higher stress resistance than those fed dried cysts throughout the experimental period, and their stress indices were significantly different after 4 weeks of feeding (Table 2). In contrast, the growth performance obtained from the group fed dried cysts was consistently higher than that of fish fed brine cysts, and the difference was significant after 2 weeks and 4 weeks of feeding. There was no significant difference in the survival rates between the two groups of cyst-fed fish.

Performance of fry of platy, swordtail, molly and tetra

During the first week of feeding, the fry of platy and swordtail showed a better feeding response to brine cysts than those of molly and tetra. Molly and tetra have a predominantly pelagic feeding behaviour during the period, and a number of fry were unresponsive to cysts sunken to the aquarium bottom. After the first week, all the fry had no difficulties feeding on brine cysts. The stress resistance of the fry

Table 2 Performance of guppy fry fed different diets

Parameters and duration of feeding	Brine cysts	Dried cysts	*Artemia* nauplii	*Moina*
Stress index (at 30‰)				
2 weeks	236.3 ± 7.32^{a}	252.5 ± 10.34^{a,b}	276.0 ± 16.79^{b,c}	291.0 ± 25.13^{c}
3 weeks	188.0 ± 5.23^{a}	208.0 ± 7.53^{a,b}	224.5 ± 5.26^{b,c}	231.5 ± 16.82^{c}
4 weeks	210.5 ± 3.11^{a}	221.8 ± 5.25^{b}	247.0 ± 6.88^{c}	253.5 ± 4.51^{c}
Wet weight (mg)^2				
2 weeks	14.3 ± 3.21^{b}	19.8 ± 2.02^{a}	15.4 ± 1.96^{a,b}	15.3 ± 0.81^{a,b}
3 weeks	52.2 ± 2.24^{a,b}	61.3 ± 11.35^{a}	45.6 ± 1.85^{b}	47.6 ± 7.04^{a,b}
4 weeks	92.0 ± 1.04^{b}	122.3 ± 4.94^{a}	91.9 ± 8.51^{b}	76.7 ± 4.71^{c}
Dry weight (mg)				
2 weeks	3.0 ± 0.77^{b}	4.4 ± 0.53^{a}	3.1 ± 0.51^{b}	3.0 ± 0.12^{b}
3 weeks	14.8 ± 0.64^{a,b}	17.7 ± 3.82^{a}	11.8 ± 0.82^{b}	12.1 ± 1.68^{b}
4 weeks	24.9 ± 1.54^{b}	37.0 ± 2.87^{a}	23.9 ± 2.51^{b,c}	19.1 ± 2.58^{c}
Total length (mm)^2				
2 weeks	12.6 ± 0.96^{b}	14.2 ± 0.52^{a}	13.1 ± 0.56^{a,b}	13.3 ± 0.21^{a,b}
3 weeks	19.3 ± 0.37^{a}	19.9 ± 1.22^{a}	18.9 ± 0.37^{a}	19.4 ± 0.97^{a}
4 weeks	20.6 ± 0.47^{b}	21.7 ± 0.46^{a}	20.2 ± 0.51^{b}	20.1 ± 0.66^{b}
Survival rate (%)				
2 weeks	67.1 ± 4.09^{a}	62.5 ± 0.91^{a,b}	59.5 ± 3.39^{b}	58.5 ± 3.79^{b}
3 weeks	65.3 ± 4.33^{a}	60.9 ± 1.31^{a,b}	58.0 ± 2.71^{b}	57.0 ± 3.72^{b}
4 weeks	65.3 ± 4.33^{a}	60.6 ± 1.11^{a,b}	58.0 ± 2.71^{b}	57.0 ± 3.72^{b}

Values are means ± standard deviations from 4 replicates. Values within each row that do not share the same superscript are significantly different ($P < 0.05$). Initial fish weight and length were 6.2 mg and 9.6 mm respectively.

of four common ornamental fish fed brine cysts and *Moina* is presented in Table 3. In swordtail, molly and tetra, the stress resistance of fry fed brine cysts was significantly higher than their corresponding groups fed *Moina*. Platy fry fed brine cysts also displayed a higher stress resistance than those fed *Moina*, although the difference was not significant after three weeks of feeding.

For growth performance in terms of wet weight, dry weight and total length, there were no significant differences between fry fed brine cysts and those fed *Moina* after 3 weeks of feeding, although brine cysts appeared to fare better in the fry of molly and tetra, while *Moina* feeding yield better results in the fry of platy and swordtail (Tables 4, 5 and 6).

Table 7 compares the cumulative survival rates of the fry of four common fish species fed on brine cysts and *Moina*. For platy and swordtail, there was no significant difference in the survival of the fry between the two diet treatments. For molly and tetra, the survival rate of fry fed brine cysts was significantly lower than that fed *Moina*. This was probably due to their poor feeding response to brine cysts during the first week of feeding. As a consequence, some mortality was observed in these two groups in the first 2 weeks. All the fry that survived the first week had no difficulties feeding on brine cysts thereafter.

A follow-up feeding experiment using tetra was conducted in four replicates for each treatment group to study whether the performance of tetra fry would improve if brine cysts are replaced by dried cysts either partially (in the first week only) or completely (throughout the experiment), and the results were compared with those of fish fed *Moina*. Table 8 showed that the survival rate of tetra fry, compared with the group fed brine cysts, improved significantly when the fry were fed dried cysts, either during the first week only or throughout the three-week experimental period. For growth performance in terms of wet weight, dry weight and total length, dried cysts appeared to fare better than brine cysts, but significant difference could only be detected in total length. On the contrary, the stress resistance of tetra fry fed dried cysts was significantly lower than that of fish fed brine cysts. It is noteworthy that in this experiment, the

Table 3 Stress resistance of the fry of platy, *Xiphophorus maculatus* (Günther), swordtail, *X. helleri* (Heckel), molly, *P. sphenops* Cuvier and tetra, *Hyphessobrycon herbertaxelrodi* Géry, in response to feeding brine decapsulated *Artemia* cysts or *Moina*

Fish and duration of feeding	Salinity used in stress test (‰)	Stress index*		Probability†
		Brine cysts	*Moina*	
Platy				
2 weeks	25	219.3 ± 32.42	285.0 ± 17.64	0.0121*
3 weeks	25	180.3 ± 37.59	226.8 ± 15.11	0.0616
Swordtail				
2 weeks	20	38.3 ± 13.13	60.5 ± 9.00	0.0317*
3 weeks	25	114.0 ± 7.44	164.3 ± 12.07	0.0006**
Molly				
2 weeks	35	215.5 ± 6.14	242.8 ± 11.70	0.0064**
3 weeks	35	143.5 ± 21.79	217.0 ± 31.11	0.0085**
Tetra				
2 weeks	15	81.0 ± 9.52	141.0 ± 19.80	0.0018**
3 weeks	15	53.5 ± 20.47	140.3 ± 23.64	0.0017**

*Values are means ± standard deviations from four replicates. †In a given row, *indicate significant difference ($P < 0.05$) while **indicate highly significant difference ($P < 0.01$) between treatments.

Table 4 Wet weight of the fry of platy, swordtail, molly and tetra fed brine decapsulated *Artemia* cysts or *Moina*

Fish and duration of feeding	Wet weight (mg)*		Probability†
	Brine cysts	*Moina*	
Platy‡			
2 weeks	16.6 ± 2.86	21.2 ± 0.91	0.0224*
3 weeks	38.3 ± 2.50	41.9 ± 8.79	0.4677
Swordtail‡			
2 weeks	27.8 ± 4.52	32.1 ± 5.32	0.2615
3 weeks	40.2 ± 5.04	45.9 ± 6.80	0.2253
Molly‡			
2 weeks	28.3 ± 1.50	29.0 ± 1.74	0.5747
3 weeks	48.7 ± 3.33	41.1 ± 5.29	0.0517
Tetra‡			
2 weeks	49.1 ± 2.79	40.9 ± 2.01	0.0033**
3 weeks	76.5 ± 6.96	69.6 ± 7.32	0.2187

*Values are means ± standard deviations from four replicates. †In a given row, *indicate significant difference ($P < 0.05$) while **indicate highly significant difference ($P < 0.01$) between treatments. ‡Initial wet weight of platy, swordtail, molly and tetra are 7.6, 8.8, 15.9 and 19.0 mg respectively.

Table 5 Dry weight of the fry of platy, swordtail, molly and tetra fed brine decapsulated *Artemia* cysts or *Moina*

Fish and duration of feeding	Dry weight (mg)*		Probability†
	Brine cysts	*Moina*	
Platy‡			
2 weeks	4.0 ± 1.10	4.6 ± 0.32	0.3183
3 weeks	7.9 ± 1.55	7.9 ± 1.93	0.9492
Swordtail‡			
2 weeks	5.1 ± 1.32	5.9 ± 0.84	0.3377
3 weeks	8.9 ± 1.28	9.4 ± 1.54	0.618
Molly‡			
2 weeks	5.8 ± 0.36	5.9 ± 0.37	0.7735
3 weeks	11.0 ± 0.444	9.0 ± 1.44	0.0376*
Tetra‡			
2 weeks	11.8 ± 0.60	9.3 ± 0.58	0.0012**
3 weeks	21.0 ± 4.04	17.1 ± 4.15	0.2253

*Values are means ± standard deviations from four replicates. †In a given row, * indicate significant difference ($P < 0.05$) while **indicate highly significant difference ($P < 0.01$) between treatments. ‡Initial dry weight of platy, swordtail, molly and tetra are 1.2, 1.5, 2.6 and 3.2 mg respectively.

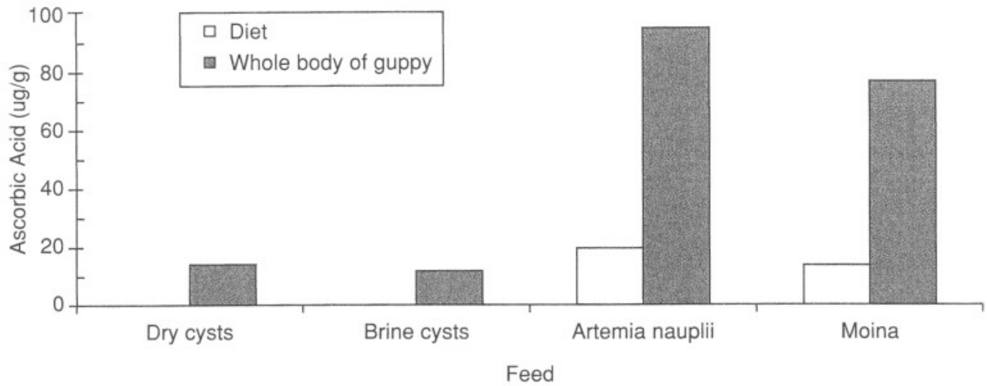

Figure 2 Comparison of the ascorbic acid content in the four diets and in the whole body of guppy, *Poecilia reticulata* Peters, fry fed the experimental diets.

Table 6 Total length of the fry of platy, swordtail, molly and tetra fed brine decapsulated *Artemia* cysts or *Moina*

Fish and duration of feeding	Total length (mm)*		Probability†
	Brine cysts	*Moina*	
Platy‡			
2 weeks	12.2 ± 0.56	13.5 ± 0.21	0.0052**
3 weeks	15.5 ± 0.62	16.3 ± 1.05	0.2094
Swordtail‡			
2 weeks	14.1 ± 0.84	15.2 ± 0.75	0.1165
3 weeks	16.4 ± 0.56	17.3 ± 0.72	0.0922
Molly‡			
2 weeks	14.2 ± 0.29	14.4 ± 0.30	0.4580
3 weeks	16.9 ± 0.39	15.9 ± 0.73	0.0529
Tetra‡			
2 weeks	19.1 ± 0.48	18.8 ± 0.23	0.2640
3 weeks	19.5 ± 0.51	19.2 ± 0.33	0.4291

*Values are means ± standard deviations from 4 replicates. †In a given row, *indicate significant difference ($P < 0.05$) while **indicate highly significant difference ($P < 0.01$) between treatments. ‡Initial total length (mm) of platy, swordtail, molly and tetra are 8.5 ± 0.15, 9.4 ± 0.34, 9.6 ± 0.28 and 12.8 ± 0.70 respectively.

growth performance of tetra fry fed either brine cysts or dried cysts was significantly better than that of fry fed *Moina*.

Ascorbic acid in diets and whole body of fry

Figure 2 compares the vitamin C content expressed in μg AA g^{-1} in the four diets used in this study and in the whole body of guppy fry fed the corresponding

Table 7 Cumulative survival rate of the fry of platy, swordtail, molly and tetra fed brine decapsulated *Artemia* cysts or *Moina*

Fish and duration of feeding	Cumulative survival rate (%)*		Probability†
	Brine cysts	*Moina*	
Platy			
1 week	99.5 ± 1.00	100.0 ± 0	0.3561
2 weeks	91.0 ± 2.16	93.8 ± 4.92	0.3461
3 weeks	87.8 ± 2.06	85.5 ± 2.08	0. 1756
Swordtail			
1 week	89.5 ± 3.70	94.5 ± 1.73	0.0500*
2 weeks	89.0 ± 3.16	91.3 ± 0.96	0.2223
3 weeks	89.0 ± 3.16	91.3 ± 0.96	0.2223
Molly			
1 week	87.0 ± 6.88	91.8 ± 0.50	0.2178
2 weeks	82.5 ± 2.52	90.5 ± 0.58	0.0010**
3 weeks	82.5 ± 2.52	90.5 ± 0.58	0.0010**
Tetra			
1 week	85.0 ± 2.71	95.0 ± 0.82	0.0006**
2 weeks	85.0 ± 2.71	94.5 ± 1.29	0.0009**
3 weeks	85.0 ± 2.71	94.5 ± 1.29	0.0009**

*Values are means ± standard deviations from four replicates. †In a given row, *indicate significant difference ($P < 0.05$) while **indicate highly significant difference ($P < 0.01$) between treatments.

diets. Among the four diets, *Artemia* nauplii recorded the highest AA content (18.9 μg g^{-1}), followed by *Moina* (12.9 μg g^{-1}). No AA was detected in dried cysts or brine cysts. The AA levels in the guppy fry were proportional to those in the diets. Guppy fed diets containing high AA exhibited a concomitant

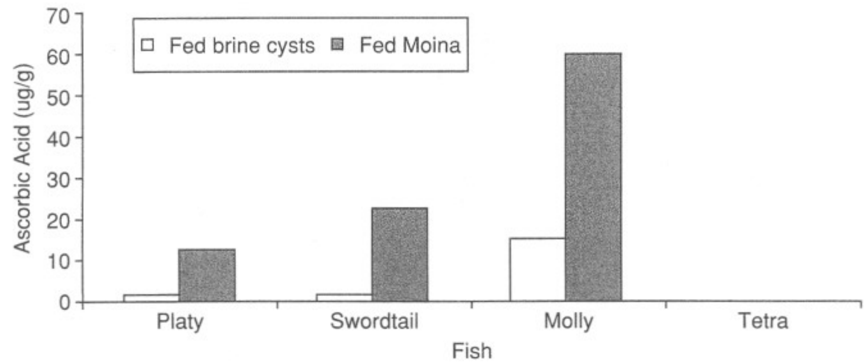

Figure 3 Comparison on the ascorbic acid content in the fry of four common ornamental fish fed brine decapsulated *Artemia* cysts or *Moina*.

Table 8 Performance of tetra fry fed different diets

Parameters and Duration of feeding	Brine cysts	Dried cysts	Dried/Brine cysts*	*Moina*
Stress index (at 15‰)				
1 week	151.0 ± 10.23^{a}	161.8 ± 13.89^{a}	189.8 ± 19.02^{b}	219.5 ± 3.11^{c}
2 weeks	43.3 ± 5.32^{a}	72.8 ± 10.53^{b}	101.3 ± 5.80^{c}	154.5 ± 7.85^{d}
3 weeks	82.3 ± 2.50^{a}	102.0 ± 3.16^{b}	85.3 ± 9.71^{a}	115.5 ± 6.81^{c}
Wet weight (mg)^{2}				
1 week	$27.9 \pm 1.86^{a,b}$	$28.6 \pm 2.25^{a,b}$	29.8 ± 2.50^{a}	25.2 ± 1.05^{b}
2 weeks	56.8 ± 3.91^{b}	67.7 ± 3.74^{a}	45.0 ± 3.49^{c}	40.5 ± 4.72^{c}
3 weeks	87.9 ± 8.78^{a}	98.4 ± 9.38^{a}	69.9 ± 3.08^{b}	62.6 ± 6.13^{b}
Dry weight (mg)^{2}				
1 week	$5.8 \pm 0.39^{a,b}$	7.0 ± 0.67^{a}	5.7 ± 0.80^{b}	4.3 ± 0.35^{c}
2 weeks	14.3 ± 1.02^{b}	18.5 ± 1.76^{a}	$12.2 \pm 0.70^{b,c}$	9.7 ± 1.07^{c}
3 weeks	24.1 ± 2.44^{a}	28.5 ± 3.14^{a}	18.2 ± 0.76^{b}	13.9 ± 1.25^{b}
Total length (mm)^{2}				
1 week	$17.2 \pm 0.41^{a,b}$	18.0 ± 0.51^{a}	$17.2 \pm 0.62^{a,b}$	16.6 ± 0.33^{b}
2 weeks	21.4 ± 0.39^{b}	22.7 ± 0.53^{a}	$20.7 \pm 0.40^{b,c}$	20.1 ± 0.72^{c}
3 weeks	22.0 ± 0.29^{b}	23.0 ± 0.33^{a}	20.9 ± 0.46^{c}	20.3 ± 0.63^{c}
Survival rate (%)				
1 week	94.8 ± 1.71^{b}	99.8 ± 0.50^{a}	100.0 ± 0^{a}	99.8 ± 0.50^{a}
2 weeks	93.3 ± 2.63^{b}	98.5 ± 1.73^{a}	98.8 ± 1.50^{a}	99.5 ± 0.58^{a}
3 weeks	92.8 ± 2.06^{b}	98.5 ± 1.73^{a}	97.5 ± 2.45^{a}	99.5 ± 0.58^{a}

Values are means ± standard deviations from four replicates. Values within each row that do not share the same superscript are significantly different ($P < 0.05$). Initial fish wet weight, dry weight and total length are 18.1 mg, 3.2 mg and 14.7 mm respectively. *Fish fed dried cysts in the first week and brine cysts in the second and third weeks

increase in the incorporated AA level and *vice versa*. Hence the AA content in guppy fry fed *Artemia* nauplii (94 $\mu g\ g^{-1}$) was higher than that of fish fed *Moina* (75 $\mu g\ g^{-1}$), which in its turn was at least five times higher than that of the fish fed dried cysts (14 $\mu g\ g^{-1}$) or brine cysts (11 $\mu g\ g^{-1}$).

Figure 3 compares the AA content in the fry of four common ornamental fish fed brine cysts or *Moina*. No AA could be detected in the tetra fry fed either brine cysts or *Moina*. In the other three fish species, the AA content in the fry fed *Moina* was always higher than those fed brine cysts. Among the fry fed *Moina*, the AA content was highest in molly (59 $\mu g\ g^{-1}$), followed by swordtail (22 $\mu g\ g^{-1}$) and then platy (12 $\mu g\ g^{-1}$). For fry fed brine cysts, the AA content in the molly was 15 $\mu g\ g^{-1}$,

Table 9 Fatty acid profiles (mg g^{-1} DW) of the four diets used in this study

Fatty acids	Brine cysts	Dried cysts	*Artemia*	*Moina*
14:0	1.3	1.5	0.3	3.1
15:0	0.3	0.4	0.2	1.7
16:0	13.7	15.8	4.1	9.4
17:0	0.9	1.1	0.3	1.3
18 : 0	4.7	5.8	3.4	3.4
Total saturates	21.2	25.2	8.9	20.0
14:1(n-5)	1.2	1.6	0.8	1.2
15:1(n-5)	0.2	0.2	0.1	0.6
16:1(n-7)	4.7	5.2	1.2	12.4
17:1(n-7)	0.6	0.6	0.0	0.5
18:1(n-9)	21.4	25.3	7.2	4.9
18:1(n-7)	8.1	9.1	3.9	6.8
20:1(n-9)	0.5	0.6	0.3	0
Total monoenes	37.0	42.9	14.0	26.8
18:2(n-6)	6.2	6.9	2.9	5.2
20:4(n-6)	0.8	1.2	1.0	1.9
Total (n-6)PUFA	7.5	8.9	4.5	7.8
Total (n-6)HUFA	1.0	1.6	1.1	2.2
18:3(n-3)	26.5	28.9	12.9	5.8
18:4(n-3)	4.1	4.2	2.1	0.3
20:3(n-3)	0.5	0.6	0.4	0.1
20:4(n-3)	0.6	0.6	0.3	0.3
20:5(n-3) EPA	4.0	4.4	0.9	2.3
22:6(n-3) DHA	0.7	1.9	0.3	0.2
Total (n-3)PUFA	36.5	41.1	17.0	9.2
Total (n-3)HUFA	6.0	8.0	2.0	3.1
DHA/EPA ratio	0.17	0.44	0.35	0.10
(n-6)/(n-3)	0.21	0.22	0.26	0.84
Total FAME	109.1	126.4	47.5	73.2

PUFA: polyunsaturated fatty acid; HUFA: highly unsaturated fatty acids (containing 20 C or more); FAME: Fatty acid methyl ester. Fatty acids were determined from a single pooled sample.

while those in swordtail (1.5 μg g^{-1}) and platy (1.4 μg g^{-1}) were almost negligible.

Fatty acid profiles of diets

The fatty acid profiles of the four diets used in this study are presented in Table 9. The most important fatty acids in the brine cysts and dried cysts were 16:0, 18:1(n-9), 18:1(n-7), 18:2(n-6), and 18:3 (n-3) and their concentrations were all higher than those in *Artemia* nauplii and *Moina*. As a consequence, the total saturates, total monoenes and total (n-3) PUFA (polyunsaturated fatty acids) in the two cyst diets were also higher than those in *Artemia* nauplii and *Moina*. In addition, the two cyst diets were also richer in 20:5(n-3) (EPA, eicosapentaenoic acid) and 22:6(n-3) (DHA, docosahexaenoic acid) and hence their total (n-3) HUFA (highly unsaturated fatty acids) were also higher than in the *Artemia* nauplii and *Moina*. For (n-6) PUFA, the cyst diets were richer in 18:2(n-6) while *Moina* was richer in 20:4(n-6). As a result, their total (n-6) PUFA was at about the same level, whereas the total (n-6) HUFA was higher in *Moina*. *Artemia* nauplii recorded the lowest (n-6) PUFA among the four diets used in this study. It

Table 10 Whole-body fatty acid profiles (mg g^{-1} DW) of guppy fry fed different diets

Fatty acids	Brine cysts	Dried cysts	*Artemia* nauplii	*Moina*
14:0	2.2	2.5	1.7	3.4
15:0	0.6	0.5	0.4	1.2
16:0	28.1	31.2	23.4	28.8
17:0	1.5	1.6	1.9	1.6
18:0	12.9	16.5	11.5	9.8
22:0	0.2	0.2	0.4	0.7
Total saturates	46.1	53.3	40.5	46.7
14:1(n-5)	1.6	1.8	1.9	0.9
15:1(n-5)	0.6	0.6	0.8	0.8
16:1(n-7)	7.1	7.7	4.0	12.4
17:1(n-7)	0.7	0.7	0.3	0.2
18:1(n-9)	38.1	48.4	23.3	12.8
18:1(n-7)	12.5	14.8	8.7	8.7
19:1(n-9)	0.5	0.5	0.8	0.2
20:1(n-9)	0.9	1.4	0.8	0.2
Total monoenes	62.3	76.3	41.1	36.2
18:2(n-6)	9.5	9.4	7.1	6.5
18:3(n-6)	0.9	1.5	1.2	1.0
20:3(n-6)	0.3	0.5	0.6	0.6
20:4(n-6)	2.1	1.8	2.5	4.7
22:4(n-6)	0.2	0.1	0.3	0.9
22:5(n-6)	0.2	0.1	0.2	1.0
Total (n-6)PUFA	13.2	13.3	12.0	14.9
Total (n-6)HUFA	2.8	2.5	3.6	7.3
18:3(n-3)	25.7	28.1	19.2	3.8
18:4(n-3)	4.7	6.2	3.9	0.4
20:3(n-3)	1.6	1.8	1.8	0.4
20:4(n-3)	1.7	3.4	2.4	0.3
20:5(n-3) EPA	2.9	3.9	1.9	0.7
21:5(n-3)	0	0	0.9	1.0
22:5(n-3)	3.3	3.8	2.6	1.7
22:6(n-3) DHA	9.3	10.4	8.5	7.3
Total (n-3)PUFA	49.7	58.2	41.7	15.5
Total (n-3)HUFA	19.2	23.9	18.6	11.4
DHA/EPA ratio	3.17	2.70	4.41	9.76
(n-6)/(n-3)	0.27	0.23	0.29	0.96
Total FAME	181.5	211.2	147.1	120.4

PUFA: polyunsaturated fatty acid; HUFA: highly unsaturated fatty acids (containing 20 C or more); FAME: Fatty acid methyl ester. Fatty acids were determined from a single pooled sample.

Table 11 Whole-body fatty acid profiles (mg g^{-1} DW) of the fry of platy, swordtail, molly and tetra fed brine decapsulated *Artemia* cysts or *Moina*

	Platy		Swordtail		Molly		Tetra	
Fatty acids	Brine cysts	*Moina*	Brine cysts	*Moina*	Brine cysts	*Moina*	Brine cysts	*Moina*
14:0	0.9	0.7	1.2	1.0	0.9	0.4	2.7	1.9
15:0	0.3	0.5	0.3	0.6	0.3	0.3	0.6	1.1
16:0	13.2	10.3	17.5	10.7	14.8	7.8	32.0	16.4
17:0	0.8	0.8	1.0	1.0	1.2	0.9	1.4	1.5
18:0	8.0	6.2	9.6	6.9	8.7	6.8	14.5	9.4
23:0	0.6	0	2.9	2.9	0.2	0	0	0.2
Total saturates	24.5	19.4	33.2	23.9	26.9	17.6	52.0	31.3
14:1(n-5)	0.7	0.3	1.1	0.4	0.9	0.2	1.6	0.6
15:1(n-5)	0.4	0.6	0.5	0.5	0.5	0.6	0.4	0.3
16:1(n-7)	3.2	3.7	4.7	4.7	4.1	1.8	9.1	11.3
17:1(n-7)	0.3	0.1	0.4	0.1	0.4	0.2	0.8	0.4
18:1(n-9)	17.9	5.8	26.5	7.3	22.7	4.7	60.5	28.0
18:1(n-7)	7.0	4.5	9.5	5.5	8.2	3.8	14.4	9.1
20:1(n-9)	0.4	0.1	1.0	1.0	0.8	0.1	0.8	0.4
22:1(n-9)	0.3	0	1.6	2.0	0	0.1	0	0
Total monoenes	30.6	15.2	46.0	21.6	38.5	11.6	88.3	50.4
18:2(n-6)	4.5	3.5	6.1	4.4	6.0	4.1	10.0	6.9
18:3(n-6)	0.7	0.4	0.9	0.7	0.7	0.5	0.5	0.3
20:3(n-6)	0.3	0.3	0.4	0.6	0.2	0.3	0.5	0.6
20:4(n-6)	2.6	3.8	2.4	3.6	3.0	5.2	1.8	2.2
22:4(n-6)	0.3	0.5	0.2	0.4	0.3	0.6	0.2	0.2
22:5(n-6)	0.5	0.8	0.4	0.8	0.7	1.4	0.2	0.4
Total (n-6)PUFA	8.9	9.3	10.5	10.5	10.9	12.0	13.2	10.6
Total (n-6)HUFA	3.6	5.3	3.4	5.3	4.3	7.5	2.7	3.4
18:3(n-3)	11.0	1.3	16.4	1.9	14.8	0.9	39.2	4.2
18:4(n-3)	2.7	0.1	4.3	0.3	2.4	0.1	4.9	0.4
20:3(n-3)	0.6	0.2	0.8	0.2	1.1	0.1	1.3	0.3
20:4(n-3)	0.7	0.1	1.7	0.3	0.7	0.1	2.2	0.3
20:5(n-3) EPA	1.6	0.6	3.1	2.0	2.9	0.6	3.4	0.6
22:5(n-3)	1.7	0.8	2.6	1.0	2.3	1.1	1.4	0.6
22:6(n-3) DHA	9.3	6.6	12.8	8.7	10.2	7.1	6.0	4.7
Total (n-3)PUFA	27.8	9.7	42.0	14.6	34.6	10.0	58.8	11.1
Total (n-3)HUFA	14.2	8.3	21.4	12.4	17.4	9.0	14.6	6.5
DHA/EPA	5.69	11.14	4.12	4.37	3.46	11.16	1.74	7.84
(n-6)/(n-3)	0.32	0.95	0.25	0.72	0.32	1.20	0.22	0.95
Total FAME	96.1	57.9	137.4	75.0	117.5	53.5	224.0	112.9

PUFA: polyunsaturated fatty acid; HUFA: highly unsaturated fatty acids (containing 20 C or more); FAME: Fatty acid methyl ester. Fatty acids were determined from a single pooled sample.

was noteworthy that *Moina* was 2.5 times richer in 16:1(n-7), than the cyst diets and 10 times richer than *Artemia* nauplii.

The DHA/EPA ratios in all four diets were below 0.5. The (n-6)/(n-3) ratios in the two cyst diets were around 0.2, which was about the same level as *Artemia* nauplii, but was only one-quarter of that recorded in *Moina*. The total FAME in the cyst diets was quite high, at least 1.5 times of that found in *Moina* and two times that in *Artemia* nauplii.

Fatty acid profiles of fry

The fatty acid profiles of guppy fry fed four different diets are given in Table 10, while those of the four other ornamental fish fed brine cysts and *Moina* are presented in Table 11. All of these profiles were similar to those of the corresponding diets fed to the fry. In all five species, the most important fatty acids in the fry fed dried cysts or brine cysts were 16:0, 18:1(n-9), 18:1(n-7), 18:2(n-6), and 18:3

(n-3). Except for 16:0 in the guppy fry, the concentrations of these fatty acids were all higher than those found in *Moina*. As a result, the total saturates (except guppy fry), total monoenes and total (n-3) PUFA in all the fry fed cyst diets were higher than those in the fry fed *Moina*. In the guppy, the 16:0 in the fry fed *Moina* was very high compared to that of the *Moina* treatment, and hence both 16:0 and total saturates were at about the same level as those recorded in fry fed the cyst diets.

As observed in the diets, all the fry fed decapsulated cysts were rich in 18:2(n-6) whereas those fed *Moina* were rich in 20:4(n-6). Hence while the total (n-6) PUFA were at about the same level, the total (n-6) HUFA in the fry fed *Moina* were higher than those fed decapsulated cysts. For (n-3) fatty acids, the fry fed decapsulated cysts were very high in 18:3(n-3), being at least six times more than those found in the corresponding fry fed *Moina*. In addition, fry fed decapsulated cysts were also rich in 18:4(n-3), EPA and DHA. Hence, both the total (n-3) PUFA and the total (n-3) HUFA in the fry fed decapsulated cysts were higher than in the corresponding species fed *Moina*. In the guppy, the total (n-6) HUFA in the fry fed *Artemia* nauplii was higher than that in fish fed decapsulated cysts, but the total (n-6) PUFA in the two groups of guppy fry was at about the same level. Both the total (n-3) PUFA and total (n-3) HUFA in guppy fry fed *Artemia* nauplii were slightly lower than those fed decapsulated cysts but higher than those fed *Moina*.

It is noteworthy that the HUFA level in the in the fry of all five species were higher than in the corresponding diets, and this was to biosyntheses of the fatty acids in the fry. In particular, the DHA levels in the fry were very high compared with the corresponding diets they were fed. As a consequence, the DHA/EPA ratios in the fry (range: 1.7–11.2) were very high compared with those measured in the diets (less than 0.4). The ratios in the fry fed *Moina* were higher than the corresponding fry fed decapsulated cysts. On the other hand, the (n-6)/(n-3) ratios recorded in the fry were at about the same levels as those detected in the corresponding diets. The ratios in the fry also followed closely with those in the diets. Hence, the ratios in fish fed decapsulated cysts were about one-third to one-quarter of those found in the corresponding fish fed *Moina*. Among the five fish species, the total FAME was highest in tetra and guppy, followed by swordtail and lowest in molly and platy. In all the five species, the total FAME in the fry fed decapsulated cysts was at least 50% more than those recorded in the corresponding fry fed *Moina*. In guppy, the total FAME in the fry fed *Moina* was lower than that fed *Artemia* nauplii.

Discussion

In this study, we demonstrated that for guppy adults and the fry of all the ornamental fish species tested, the performance in terms of growth, survival and stress resistance of fish fed decapsulated *Artemia* cysts was better than or similar to those fed *Artemia* nauplii or *Moina*. The only negative result of cyst-feeding observed in this study was the lower survival rates recorded in molly fry and tetra fry that were fed brine cysts, compared with those fed *Moina*. This was at least partly due to the rapid settling of the brine cysts, which made them less accessible to the pelagic fry during the first week of feeding. Hence more buoyant dried cysts should be used for feeding pelagic fish fry. Our follow-up experiment using tetra fry showed that the survival rate was improved significantly when the fry were fed dried cysts, either during the first week only or throughout the 3-week experimental period (Table 8). When comparing the results of the two experiments using guppy fry (Table 2) and tetra fry, respectively (Table 8), it became apparent that fish fed brine cysts tended to display a higher level of stress resistance, while those fed dried cysts tended to fare better in growth and survival. The better growth recorded in fry fed dried cysts corroborated the observation that heat treatment at 40 °C used in cysts preparation would not affect the protein quality of the cysts (Garcia-Ortega, Verreth, Van Hoornyck & Segner 2000).

The higher stress resistance in fish fed brine cysts compared with those fed dried cysts was not associated with the AA content, as both brine cysts and dried cysts were deficient in AA (Fig. 2). Similarly, for all the five fish species tested, the higher stress resistance recorded in fry fed decapsulated cysts compared with those fed *Moina* (and guppy fry fed *Artemia* nauplii) could not be attributed to the AA content, because of their lower AA content. The low AA content in the cyst-fed fry were expected because vitamin C in decapsulated *Artemia* cysts was in the form of ascorbic acid 2-sulphate (AAS), which is of low bio-availability to fish (Ashraf, Simpson, Bengtson, Barrows & Maugle 1996; Van Stappen 1996; Amerio, Ruggi, Rovelli & Volker 1998). It has been established that AA deficiency in fish can

result in scoliosis, lordosis and macrocytic anaemia (Halver 1970). Such syndromes were not observed in the cyst-fed fry of all of the five ornamental fish species. The dietary AA requirement of these fish species is a subject of future research.

An important consideration of the nutritional quality of fish diets is their fatty acid composition. Linolenic acid, 18:3(n-3), is widely considered to be an essential fatty acid (EFA) for freshwater organisms and EPA an EFA for marine organisms (Kanazawa, Teshima & Ono 1979). Watanabe (1987) reviewed the EFA requirement of freshwater and marine fish and concluded that freshwater species required mainly 18:2(n-6) or 18:3(n-3) or both as EFA. He suggested that the (n-3) HUFA, the EFA for marine fish, was also very effective in the diets of freshwater fish. Examination of the fatty acid profiles has revealed that dried cysts and guppy fry fed dried cysts are richer in (n-3)HUFA, including both EPA and DHA, than brine cysts and fish fed brine cysts respectively (Tables 9 and 10). The lower contents in brine cysts could be due to their further metabolism after processing, as they were still alive while the dried cysts were dead. Tuncer & Harrell (1992) reported that in the larval striped bass, *Morone saxatilis* (Walbaum), and palmetto bass, *M. saxatilis* × *M. chrysops* (Rafinesque), the higher the HUFA content in the diet and larvae, the better the survival and growth. Furuita, Takeuchi, Toyota & Watanabe (1996) found that the juvenile red sea bream, *Pagrus major* (Temminck & Schlegel), fed EPA-enriched *Artemia* had high survival rates but poor vitality, while those fed *Artemia* enriched with DHA had both a high survival rate and vitality. These data suggested that the better growth and survival rate obtained in guppy fry (and probably tetra fry) fed dried cysts compared with those fed brine cysts could be due to the higher HUFA, EPA and DHA contents in the dried cysts.

The fatty acid profiles revealed that the two cyst diets were richer in EPA and DHA, and hence their total (n-3) HUFA was similarly higher than the *Artemia* nauplii and *Moina*. The total FAME in the cyst diets was at least 50% more than that found in *Moina* and 100% more than that in *Artemia* nauplii. These results suggested that the fatty acid composition of decapsulated cysts, both in quality and quantity, was superior to those in *Artemia* nauplii and *Moina*. The better performance in fish fed decapsulated cysts could at least be partly attributed to the superior fatty acid composition of the cysts, which corresponded to the higher energy content in *Artemia* cysts than in their nauplii (Vanhaecke, Lavens & Sorgeloos 1983). In particular, the higher stress resistance observed in cyst-fed fry could be associated with their higher (n-3)HUFA, which is also known to reduce the effects of stress (Menasveta 1994). These results may also imply that when fry are given more adequate energy levels in their feeds, they will not only grow and survive better, but may also have better stress resistance.

From the forgoing results, it became clear that the performance of fish fed decapsulated *Artemia* cysts was better than or similar to those fed *Artemia* nauplii or *Moina*. These results implied that for fry that could readily accept inert diets, decapsulated *Artemia* cysts could be used as a complete substitute for *Artemia* nauplii or *Moina* in ornamental fish culture. The use of decapsulated cysts would offer the following advantages to the industry:

a. More hygienic feed: All decapsulated cysts are disinfected by hypochlorite during the decapsulation process. They are therefore more hygienic than the potentially contaminated *Moina*.
b. Off-the-shelf feed: Decapsulated cysts can be processed for long-term storage, and may be fed to fish directly as and when required. This characteristic would ensure a ready supply of the feed to farms, and also the possibility of more frequent food distribution to the culture tanks. The reduced feed retention time in the culture tank would lead to better water quality and lower risk of nutritional deterioration of the feeds.
c. Lower *Artemia* cyst requirement: Decapsulated cysts constitute the highest energy form of *Artemia* and hence a lower amount of cysts would be required to provide same energy level as the nauplii. The improved energy balance of the decapsulated cysts may also result in substantial saving in feed cost.
d. Labour saving: Compared with the use of *Artemia* nauplii, direct feeding of decapsulated cysts would alleviate the heavy work load in hatchery operation, as the labour intensive nauplii production is no longer necessary.

It has been estimated that about 50% of the *Artemia* cysts stocks have a low commercial value due to their low hatchability (Leger, Bengtson, Simpson & Sorgeloos 1986). These cysts can be acquired cheaply, at a fraction of the cost of the regular *Artemia* cysts (estimated to be US\$ 6 kg^{-1} vs. US\$ 60 kg^{-1}). After decapsulation and dehydration, the weight of decapsulated cysts is reduced by 46% (Verreth & Bieman 1987), but this would not affect

the utilizable amount of the cysts. Taking into consideration the cost of processing ($\$ 12\,\mathrm{kg}^{-1}$) for decapsulated cysts and cost of hatching ($\$ 6\,\mathrm{kg}^{-1}$) for regular cysts, the decapsulated cysts were estimated to cost 27% ($\$ 18\,\mathrm{kg}^{-1}$ vs. $\$ 66\,\mathrm{kg}^{-1}$ based on original weight of the cysts) of the regular *Artemia* cysts. For regular cysts, only a portion of cysts is hatched into nauplii for feeding, whereas for decapsulated cysts, all the cysts can be used directly for feeding. When compared with an average batch of cysts with 75% hatching percentage, the decapsulated cysts was estimated to cost 20% (27% × 75%) of *Artemia* nauplii produced in the fish hatchery. As the dry weight and energy content of decapsulated cysts are 30–40% higher than for instar 1 nauplii (Van Stappen 1996), the cost of decapsulated cysts is further reduced to 15% (20% divided by 1.35) of the *Artemia* nauplii in terms of feeding efficiency.

In conclusion, the availability of the low cost and more hygienic decapsulated *Artemia* cysts will provide the ornamental fish industry with a suitable feed substitute for *Artemia* nauplii or *Moina*. The direct use of decapsulated cysts may also open a new area of application for low-hatch or no-hatch cysts in the ornamental fish industry, and hence a substantial saving in the feed cost. Use of the decapsulated cysts is likely to have a positive impact in terms of farm hygiene and feed cost to the ornamental fish industry.

Acknowledgments

This work was supported by the Agri-food and Veterinary Authority of Singapore (Project FFC/E6/99).

References

Amerio M., Ruggi C., Rovelli R.M. & Volker A. (1998) Ascorbic acid availability from ascorbyl 2-polyphosphate and ascorbyl 2-sulfate in sea bass (*Dicentrarchus labrax*). *Aquaculture* **159**, 233–237.

Ashraf M., Simpson K.L., Bengtson D.A., Barrows R. & Maugle P.D. (1996) Effect of dietary ascorbic acid vitamin supplements on growth and survival of striped bass, *Morone saxatilis, and* inland silverside, *Menidia beryllina*, larvae. *Pakistan Journal of Zoology* **28** (3), 185–189.

Bardocz T., Kovacs E., Radics F. & Sandor Z. (1999) Experiments for the improved use of decapsulated *Artemia* cysts in intensive culture of African catfish larvae. *Journal of Fish Biology* **55**, 227–232.

Bengtson D.A., Leger P. & Sorgeloos P. (1991) Use of *Artemia* as a food source for aquaculture. In: *Artemia Biology* (ed. by R. A. Browne, P. Sorgeloos & C. N. A. Trotman), pp. 255–285. CRC Press, Boca Raton, FL, USA.

Bruggeman E., Sorgeloos P. & Vanhaecke P. (1980) Improvements in the decapsulation technique of *Artemia* cysts. In: *The Brine Shrimp Artemia*, Vol. 3. *Ecology, Culturing, Use in Aquaculture* (ed. by G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers), pp. 261–269. Universa Press, Wetteren, Belgium.

Coutteau P. & Sorgeloos P. (1995) Intercalibration exercise on the qualitative and quantitative analysis of fatty acids in *Artemia* and marine samples used in mariculture. *ICES Cooperative Research Report*, no. 211. International Council for the Exploration of the Sea, Denmark.

De Los Santos C. Jr, Sorgeloos P., Lavina E. & Bernardino A. (1980) Successful inoculation of *Artemia* and production of cysts in man-made salterns in the Philippines. In: *The Brine Shrimp Artemia*, Vol. 3. *Ecology, Culturing, Use in Aquaculture* (ed. by G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers), pp. 159–163. Universa Press, Wetteren, Belgium.

Furuita H., Takeuchi T., Toyota M. & Watanabe T. (1996) EPA and DHA requirements in early juvenile red sea bream using HUFA enriched *Artemia* nauplii. *Fisheries Science* **62**, 246–251.

Garcia-Ortega A., Verreth J., Van Hoornyck A. & Segner H. (2000) Heat treatment affects protein quality and protease activity in decapsulated cysts of *Artemia* when used as starter food for larvae of African catfish *Clarias gariepinus* (Burchell). *Aquaculture Nutrition* **6**, 25–31.

Halver J.E. (1970) Nutrition in marine aquaculture. In: *Marine Aquaculture* (ed. by W.J. McNeil), pp. 75–102. Oregon State University Press, Corvallis, OR, USA.

Kanazawa A., Teshima S.I. & Ono K. (1979) Relationship between fatty acid requirements of aquatic animals and the capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. *Comparative Biochemistry and Physiology* **63B**, 295–298.

Leger P., Bengtson D.A., Simpson K.L. & Sorgeloos P. (1986) The use and nutritional value of *Artemia* as a food source. *Oceanography and Marine Biology Annual Review* **24**, 521–623.

Lepage G. & Roy C.C. (1984) Improved recovery of fatty acid through direct transesterification without prior extraction or purification. *Journal of Lipid Research* **25**, 1391–1396.

Lim L.C., Soh A., Dhert P. & Sorgeloos P. (2001) Production and application of on-grown *Artemia* in freshwater ornamental fish farm. *Aquaculture Economics and Management* **5**, 211–228.

Lim L.C., Wong C.C., Koh C.H., Dhert P. & Sorgeloos P. (2000) A stress resistance test for quality evaluation of

guppy (*Poecilia reticulata*) (abstract). In: *Abstract Book of 1st AVA Technical Seminar, 1 September 2000*, pp. 4–5. Agri-Food and Veterinary Authority, Singapore.

Menasveta P. (1994) Dietary prophylaxis in fish and shrimp. In: *Proceedings of the 1st Roche Aquaculture Centre Conference on Nutritional Prophylaxis, 23 March 1994, Bangkok*. Rovithai, Thailand.

Mock C.R., Fontaine C.T. & Revera D.B. (1980) Improvements in rearing larval penaeid shrimp by the Galveston Laboratory method. In: *The Brine Shrimp Artemia*, Vol. 3. *Ecology, Culturing, Use in Aquaculture* (ed. by G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers), pp. 331–342. Universa Press, Wetteren, Belgium.

Nelis H., Merchie G., Lavens P., Sorgeloos P. & De Leenheer A. (1997) Liquid chromatographic determination of vitamin C in aquatic organisms. *Journal of Chromatographic Science* **35**, 337–341.

Pector R., Tackaert W., Abelin P., Ollevier F. & Sorgeloos P. (1994) A comparative study on the use of different preparations of decapsulated *Artemia* cysts as food for rearing African catfish (*Clarias gariepinus*) larvae. *Journal of the World Aquaculture Society* **25**, 366–370.

Ribeiro F.A.L.T. & Jones D.A. (1998) The potential of dried, low hatch, decapsulated *Artemia* cysts for feeding prawn post-larvae. *Aquaculture International* **6**, 421–440.

Royan J.P. (1980) Importance of *Artemia salina* as food in shrimp culture. *Abstracts of the Symposium on Coastal Aquaculture, 12–18 January 1980*, pp. 133. Cochin, India.

Sorgeloos P., Bossuyt E., Lavens P., Leger P., Vanhaecke P. & Versichele D. (1983) The use of brine shrimp *Artemia* in crustacean hatcheries and nurseries. In: *CRC Handbook or Mariculture*, Vol. 1. *Crustacean Aquaculture* (ed. by J.P. McVey & J.R. Moore), pp. 71–96. CRC Press, Boca Raton, FL, USA.

Sorgeloos P., Bossuyt E., Lavina E., Baeza-Mesa M. & Persoone G. (1977) Decapsulation of *Artemia* cysts: a simple technique for the improvement of the use of brine shrimp in aquaculture. *Aquaculture* **12**, 311–316.

Sorgeloos P., Lavens P., Leger Ph, Tackert W. & Versichele D. (1986) *Manual for the culture and use of brine shrimp Artemia in aquaculture. Artemia* Reference Center, Faculty of Agriculture, State University of Ghent, Ghent, Belgium.

Tuncer H. & Harrell R.M. (1992) Essential fatty acid nutrition of larval striped bass (*Morone saxatilis*) and palmetto bass (*M. saxatilis* x *M. chrysops*). *Aquaculture* **101**, 105–121.

Van Stappen G. (1996) Use of cysts. In: *Manual on the Production and Use of Live Food for Aquaculture* (ed. by P. Lavens & P. Sorgeloos), pp. 107–136. FAO Fisheries Technical Paper, no. 361, FAO, Rome.

Vanhaecke P., Lavens P. & Sorgeloos P. (1983) International study on *Artemia*. XVII. Energy consumption in cysts and early larval stages of various geographical strains of *Artemia*. *Annales de la Société Royale Zoologique de Belgique* **113**, 155–164.

Verreth J. & Bieman H.D. (1987) Quantitative feed requirements of African Catfish (*Clarias gariepinus* Burchell) Larvae fed with decapsulated cysts of *Artemia*. I. The effect of temperature and feeding level. *Aquaculture* **63**, 251–267.

Verreth J., Storch V. & Segner H. (1987) A comparative study on the nutritional quality of decapsulated *Artemia* cysts, micro-encapsulated egg diets and enriched dry feeds for *Clarias gariepinus* (Burchell) larvae. *Aquaculture* **63**, 269–282.

Watanabe T. (1987) The use of *Artemia* in fish and crustacean farming in Japan. In: *Artemia Research and its Applications*, Vol. 3 *Ecology, Culturing, Use in Aquaculture* (ed. by P. Sorgeloos, A. Bengtson, W. Decleir & E. Jaspers), pp. 372–393. Universa Press, Wetteren, Belgium.